Clonal Trees in the Bioeconomy Age: Opportunities and Challenges

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Use of liquid medium and biofortificants for improving micropropagation and acclimation of *Musa* AAA cv. Williams

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In Ecuador, banana production is being threatened by the age of plantations and diseases like *Mycosphaerella fijiensis* Morelet.
Although bananas are traditionally propagated by suckers, by using tissue-cultured plants it is possible to produce larger quantities of healthy plantlets for both small- and large-scale farmers.

In this study, several genotypes of banana growing at "El Oro" (Ecuador) were established *in vitro*, propagated and acclimated.
Here we describe three approaches that allowed the successful micropropagation of these bananas.

CULTURE ESTABLISHMENT

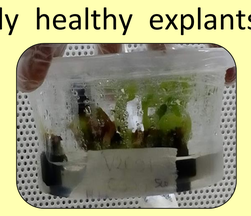
Two modifications were incorporated to the conventional sterilization method, in order to ↓ exogenous and endogenous contaminants:

1) **Mechanical shaking**: Explants were vigorously shaken using a commercial device during the first sterilization step outside the laminar flow cabinet.


2) **Wounding**: After 14 days of their inoculation in the culture medium (MS BA 2 mg/L + IBA 0.023 mg/L + sucrose 3%), two perpendicular incisions were made to the surviving explants, to detect latent contamination. In this way, only healthy explants were selected for initiation of cultures.







PROPAGATION IN LIQUID MEDIUM BY TEMPORARY IMMERSION (TIS)

Banana explants were cultured in MS BA 2 mg/L + IAA 0.65 mg/L + sucrose 3% in semisolid medium with agar (**jars**) and in liquid medium by **temporary immersion**


Commercial and **in-house** bioreactors were programmed for 8 immersions per day (4 min each).

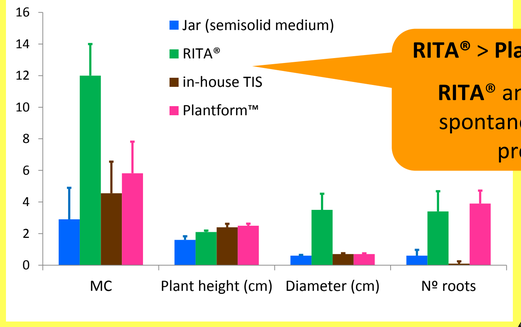
Container	Volume of medium (ml)	Nº of explants
Jar	200	4
RITA®	200	4
In-house TIS	1000	20
Plantform™	500	10

Control: explants in jars

Commercial bioreactor Plantform™

Commercial bioreactor RITA®

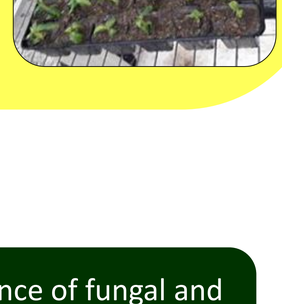
In-house TIS bioreactors (double flask system)



RITA® > Plantform™>in-house TIS ≥ jar

RITA® and **Plantform™** produced spontaneously rooted explants in proliferation medium

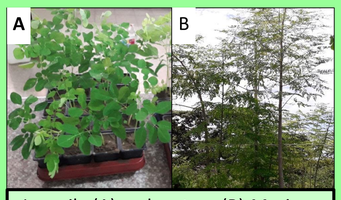
Rooted plants were successfully acclimated



ACCLIMATION WITH A BIOFORTIFIER

Vitroplants were sprayed with extracts of *Moringa oleifera*, a tree with biofortificant properties.

- Banana vitroplants were acclimated for 3 weeks in a greenhouse.
- Then they were sprayed with extracts of leaves of juvenile and mature *Moringa*, a tree with high biotechnology potential as growth enhancer and plant defense promoter.

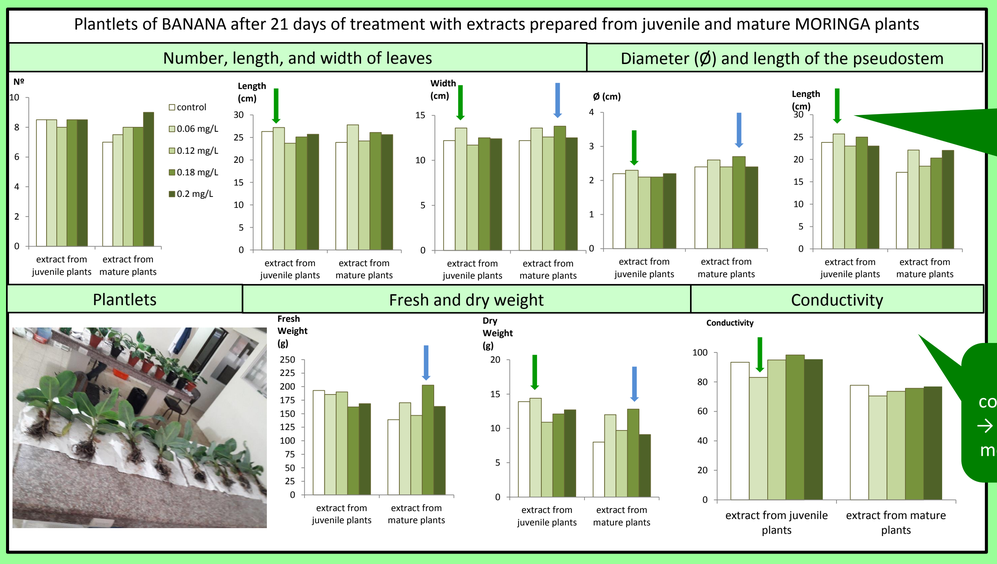


Juvenile (A) and mature (B) *Moringa* plants used for extract preparation


Moringa leaves were dried, dissolved in water and filtered



- Growth parameters were recorded 3 weeks later



Extracts with 0.06 mg/L of leaves from juvenile plants and 0.18 mg/L of leaves from mature plants improved acclimation of banana vitroplants

Low conductivity → functional membranes

The obtained results indicate: i) the need to follow extremely careful sterilization protocols for establishing banana cultures, in order to counteract the presence of fungal and bacterial contaminants due to the climate conditions in "El Oro" (Ecuador), ii) the potential of temporary immersion systems for propagation of banana, and iii) the beneficial effects of extracts of *Moringa* leaves on vitroplants of banana during the acclimation step.

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